Lipids Metabolism in the Cerebral Edema Glycolipids Metabolism in the Experimentally Produced Cerebral Edema

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by

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It is well known that the brain contains large amount of lipids compared with other tissues. However, their physiological meanings had been left almost unknown untill recently.

During the last ten years, various informations on cerebral lipids have been accumulated and it has become clear that they act not only as an inert structural constituent but they intimately concern with the functional mechanism[1)2)7)31)38)39)40)70)71)72)73)101)107)] of the nervous system.

It is often observed that brain swelling or edema which occures secondarily to various pathological conditions of the brain, e. g. head injuries, brain tumors, vascular lesions, cerebral intoxications of endogenous or exogenous nature, and infectious processes, produces many serious neurological symptoms or signs and can even be the causes of fatal sequelae. However, the inherent mechanism of cerebral edema or swelling has not been well clarified yet.

Previous investigations[95)] studied by means of the electron microscope in this laboratory demonstrated some derangement of periodicity and disappearence of interperiodic layers of myelin sheath, and elective demyelination in the experimentally produced edematous brains of the cat. Also HOLTET[42)43)] disclosed a reversible chromatolytic changes[78)] of the nerve cell with enormous swelling of cytoplasm in the experimentally produced brain edema in rats.

Of the two important kinds of glycolipids in the nervous tissue, cerebrosides[9)47)53)69)] are the important constituent of the myelin lipids[9)] together with sphingomyelin, cholesterol and phosphatidyl serine. They appear relatively late either in phylogenetical[69)85)] or ontogenetical[12)30)] evolution of the brain, i. e. they are virtually absent at birth and are deposited gradually in the course of myelination of the nerve fibers[90)]. While, gangliosides[6)55)57)] in the brain are found mainly in the grey matter[54)56)62)], especially in the microsomal fractions which contain fragments of cell membranes, particularly those of dendrites[106)]. They are present in the brains at birth[90)]. Different from many other lipids, gangliosides are acidic, acid-sensitive, non-dialysable and water-soluble.

The aim of the present investigation was to study the metabolic changes of these two lipids during the course of cerebral swelling and to seek for some available information for the treatment of the cerebral traumatism.

EXPERIMENTAL PROCEDURE

For the production of cerebral swelling in the experimental animals, right frontal lobe of the adult cats weighing 3.0 to 4.0 kg was compressed for 24 hours by the inflation of the rubber balloon which had been inserted epidurally. Cerebral compression was carried out under the E. E. G. control so as to standardize the degree of insultation. The operation was performed with full aseptic precautions under ether anesthesia. Details of the technique of produceing cerebral swelling had been described previously[44)45)58)].

Animals were sacrificed at immediately, 24 and 48 hours after evacuation of balloon content respectively. The compressed portion of the brain was removed immediately after exsanguination and was frozen, using dry-ice. At the same time, a corresponding portion of the side opposite to the compression was removed to serve as a control. Frozen material was stored at −20°C for the following examination.

The brain was divided carefully into grey matter, subcortical white matter and brain-stem after removal of pial vessels.

Each sample was weighed on a torsion balance after removal of free water, by blotting with a bit of filter paper (Toyo-roshi No. 1).

An aliquot of tissue sample was homogenized for three minutes in a Potter-Elvehjem type of homogenizer with cold (4°C) 2 : 1 chloroform-methanol mixtures (v/v) to a final dilution 20 fold the volume of tissue sample.

Extraction was carried out three times with solvent mixtures and then subjected to solvent partition as described by FOLCH, et al[29)]. The procedure used is outlined in Figure 1.

For the removal of non-lipids contamination and to separate gangliosides from cerebrosides, the crude lipid extract was washed with 0.2 volume of 0.37 per cent potassium chloride. The mixture was allowed to separate into two phases by centrifugation at 2,400 r. p. m. for twenty minutes. The lower phase was washed twice with 0.4 volume of *pure solvent upper phase*, a mixture of chloroform, methanol and 0.74 per cent potassium chloride in the proportions of 3 : 48 : 47 by volume.

Besides for these chemical assey a part of tissue aliquot was left for measurement of dry weight.

Determination of cerebrosides.

Cerebrosides were estimated by their galactose moiety which was released by hydrolysis in hydrochloric acid.

An aliquot of lower phase which had been made to standard volume and contained 10 mg of grey matter in wet weight or 2 mg of white matter in wet weight was evaporated to dryness under reduced pressure.

After the samples were hydrolysed[3)] in 3 N hydrochloric acid for 45 minutes in a boiling water bath, fatty acids were removed by centrifugation and glycerol was removed by washing with ice-cold ethyl acetate according to EDMAN[20)].

Galactose was estimated by carbazol reaction of DISCHE[18)19)] with some modification by GURIN and HOOD[37)]. Optical densities at 540 mμ were measured by the KLETT-SUMMERSON's colorimeter.

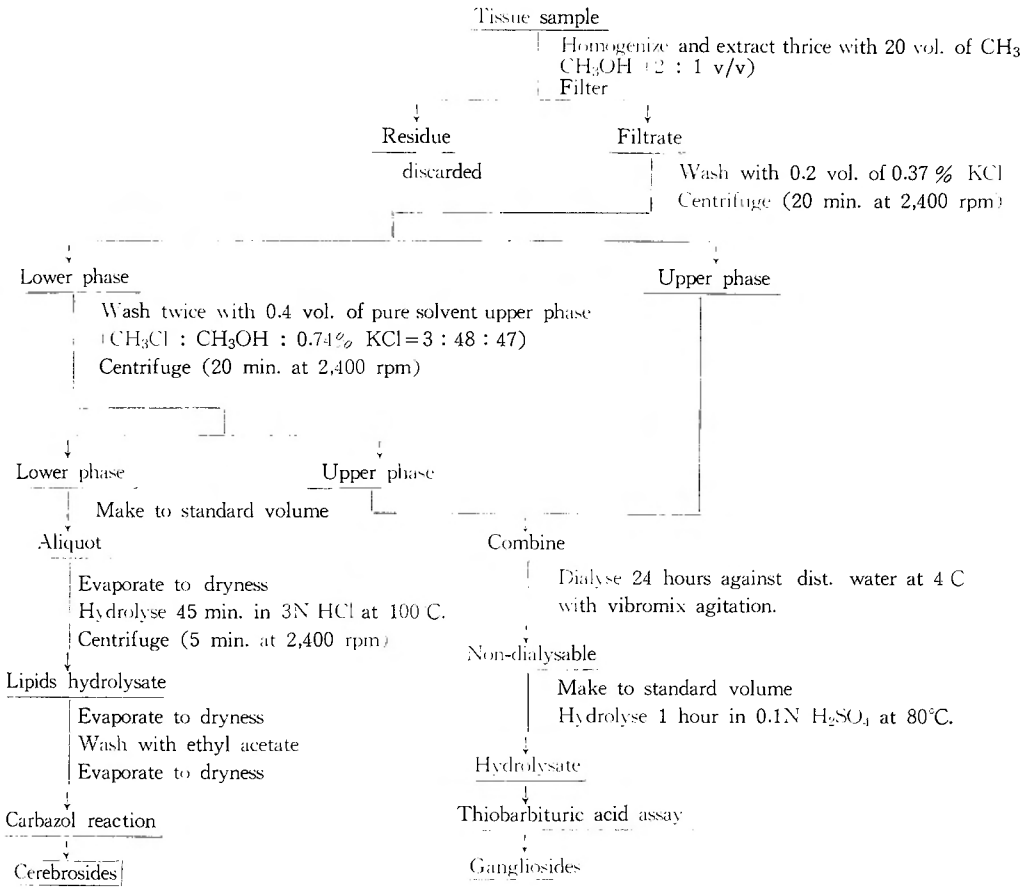


Figure 1.

Determination of gangliosides.

Gangliosides were estimated by their N-acetyl neuraminic acid moiety which was released by hydrolysis in aqueous sulfuric acid.

The three upper phases obtained from above mentioned procedures (cf. Figure 1.) were combined and concentrated almost to dryness, then dialyzed for 24 hours against distilled water at 4°C in a 0.25 in. diameter Visking sack in cylindrical jars of approximately 10 l. capacity, with four changes of water, being agitated by a magnetic stirrer. After dialysis, the sacks were emptied and rinsed and the contents were made to a standard volume. An aliqot (0.4 ml.) of the dialyzed upper phase obtained from 50 mg of grey matter in wet weight was hydrolysed in 0.1N sulfuric acid for one hour at 80°C in a water bath. Determinations of N-acetyl neuraminic acid content were carried out according to WARREN's thiobarbituric acid method[46)103)]. Optical densities at 532 and 549 mμ were determined by a BECKMANN's electrophotometric colorimeter. The formula μM of N-acetyl neuraminic acid = 0.09 OD_{5490} - 0.033 OD_{5320} was used to correct for 2-deoxyribose contamination, though it was almost negligible. N-acetyl neuraminic acid from horse erythrocytes, obtained from Takeda Pharmaceutical Co., Ltd., was used as a standard.

Determination of dry weights of tissue samples.

For the purpose of determining dry weights of tissue samples small flat bottomed vials measuring approximately 10 mm in length and 8 mm in diameter and weighing about 500 mg were used as containers. The tissue samples of about 100 mg wet weight were dried for two and half hours at a temperature of 105–110°C in an oven. Samples were weighed before and after drying.

All analysis described here were done in duplicate, and experiments with difference in values between duplicate samples greater than 8 per cent were discarded.

Application of uridine derivatives for the treatment of animals with cerebral trauma.

Uridine derivatives, including uridine, UTP and UDP-glucose were given for the therapeutic purpose to the animals with cerebral traumatism produced by the epidural cerebral compression method which had been described above. Drugs were given in a dosis of 60 mg (20 mg/kg body weight) intravenously through a poly-ethylene tube which had been inserted through the right external jugular vein previously.

RESULTS

Figures 2 and 3 and Table 1 give the concentration of cerebrosides in the subcortical white matter, brain-stem and grey matter in normal and edematous brains. In Table 1, results are expressed as mean ± standard error of mean ($P = 0.05$). The concentration of cerebrosides in the subcortical white matter, brain-stem and the grey matter of the normal specimens are 5.27 ± 0.48, 3.40 ± 0.39 and 0.84 ± 0.11 mg/100 mg wet weight respectively, being well accorded to the values reported by the others[47]. In white matter, the amounts of cerebrosides in the specimens of 0 and 24

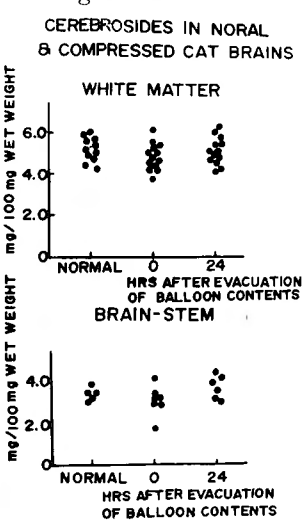


Figure 2

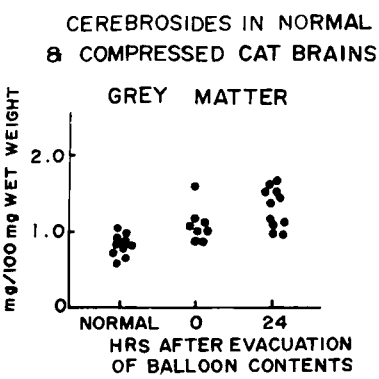


Figure 3.

Table 1. Amounts of cerbrosides in the grey matter, white matter and brain-stem of normal and edematous cat brain, being expressed as mg/100 mg fresh weight. Results are given as mean ±S. E. M Number of animals examined are shown in parenthesises.

		Grey matter	White matter	Bsain-stem
Normal		0.84±0.11 (10)	5.27±0.48 (10)	3.40±0.39 (5)
Edematous	0 hour specimens	1.07±0.15 (8)	4.83±0.35 (13)	3.07±0.71 (7)
	side opposite	—	4.92±0.42 (7)	—
	24 hours specimens	1.30±0.13 (11)	5.10±1.04 (13)	3.69±0.61 (6)
	side opposite	—	5.28±0.82 (7)	—

hours periodes after evacuation of balloon content are 4.83±0.35 and 5.10±1.04 on the compressed side, 4.92±0.42 and 5.28±0.82 on the side opposite to the compression respectively. No significant changes are found in these values when compared with those of normal brain or with those of the opposite side in each brain specimen. In the brain-stem, they are 3.07±0.71 and 3.69±0.61 for 0 and 24 hours specimens and again no significant shift from normal values is noticed. For the grey matter, however, concentration of cerebrosides in 0 and 24 hours specimens are 1.07±0.15 and 1.30±0.13 respectively, and slight increment of these values from normal specimens is noted.

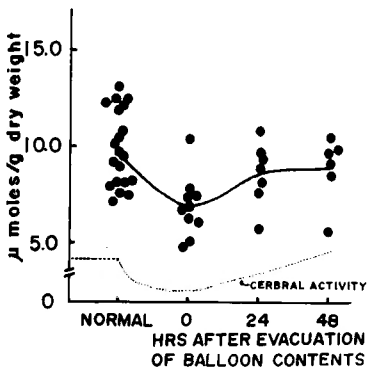


Figure 4.

The concentration of gangliosides of grey matter in normal and edematous brain are given in Figure 4 and Table 2. Their amounts for normal speciemns are 9.28±1.84 μ moles/g dry weight and are similar to those reported by the others[56)60)]. Those values for 0, 24 and 48 hours specimens are 6.81±0.99, 8.55±1.11 and 8.85±2.07 respectively. Here is significant decrease of their amounts in 0 hour specimens, when the cerebral activity as was expressed by E. E. G. tracing and clinical symptoms was maximally deteriorated. With restoration of cerebral activity, after removal of the content of the epidural balloons, the concentration of gangliosides increased to normal level within 48 hours.

Table 2. Amounts of gangliosides in normal and edematous cat brain. (μ moles/g dry weight) Results are expressed as mean ±S.E.M. (P=0.05) Number of animals examined are shown in parenthesises.

Normal		9.28±1.84 (20)
Edematous	0 hour specimens	6.81±0.99 (10)
	24 hours specimens	8.55±1.11 (7)
	48 hours specimens	8.85±2.07 (6)

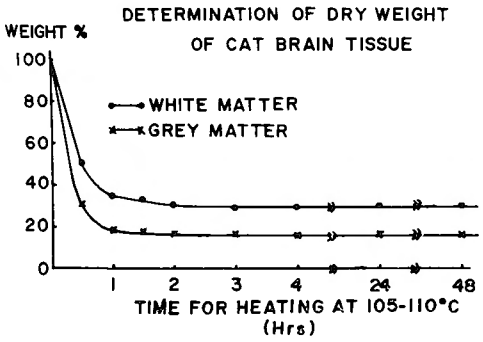


Figure 5.

Figure 5 shows the changes of weight of grey matter and white matter specimens during heating at a temperature of 105–110°C in an oven. The values are expressed by per cent of initial weight. It shows a decrement curve of tissue weight which arrives to a plateau within two hours both in the grey and the white matter. It is clear from the

Table 3. Dry weight/wet weight per cent of grey matter and white matter of normal and edematous cat brain. Mean ± S. E. M. (P=0.05) of seven cases are shown respectively.

		Grey matter	White matter
Normal		19.5±1.1	31.6±2.1
Edematous	0 hour specimens	19.3±1.5	32.0±2.0
	24 hours specimens	20.2±1.8	30.7±2.3
	48 hours specimens	21.1±1.7	—

figure that two and a half hours are enough to dry up the specimen. Table 3 shows dry weight wet weight per cent of grey matter and subcortical white matter of normal and compressed cat brains. Normal values for grey matter and white matter are 19.5±1.1 and 31.6±2.1 respectively. No significant changes are obtained from the table during the course of epidural compression, when free water of tissue were eliminated by blotting with a bit of filter paper before determination.

Uridine derivatives were given to the brain damaged animals intravenously for the therapeutic purpose. Figures 6 and 7 show restoration of cerebral activity as was expressed in E. E. G. tracing after the administration of uridine, 60 mg (20 mg/kg body weight) intravenously for the drowsy cats with the brain traumatism of the fairly constant degree. Before administration of the drug, E. E. G. displayed high voltage slow bursts (3-8 cps) in the bilateral leads, predominant on the compressed side. Three to five minuts after injection of the drug, high voltage slow waves began to disappear and nearly normal E. E. G. with alpha activity appeared after ten to fifteen minutes. These effects persisted for forty to fifty minutes following the injection. The effects of uridine were reproduceable and similar results were obtained with another three cats.

Administrations of UTP and UDP-glucose were also tried to the brain traumatized animals in the dosis of 20 mg/kg body weight. These drugs were, however, less effective in restoring cerebral activity than uridine of the same dosis. Improvement of E. E. G. findings was also noticed in these cats but it persisted only for ten minutes or slightly longer.

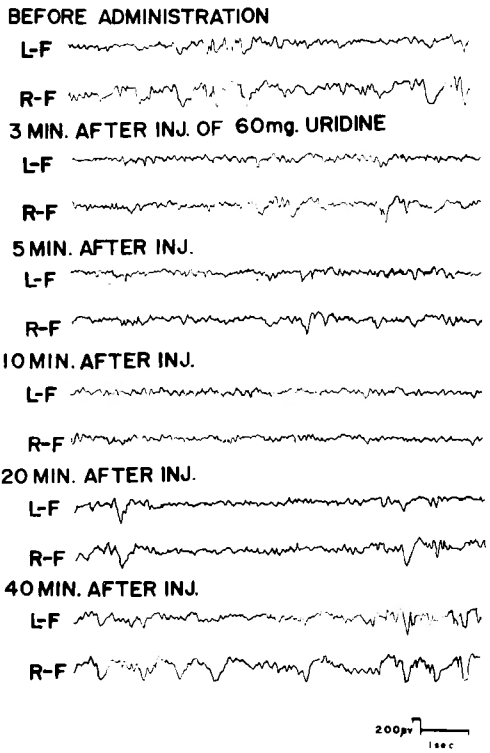


Figure 6.

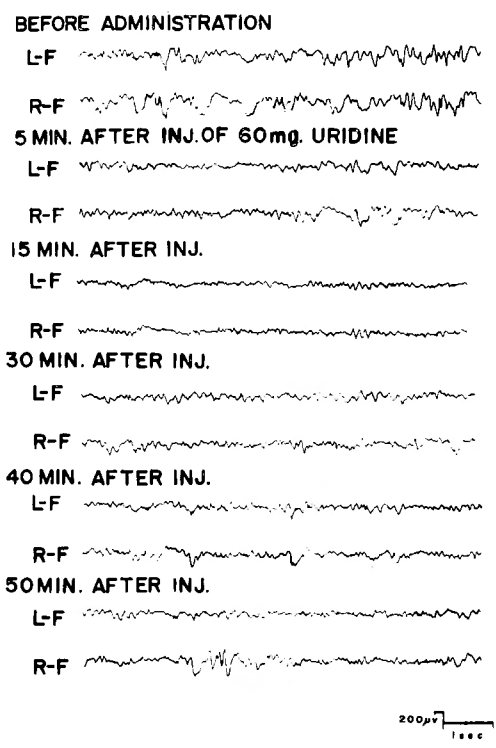
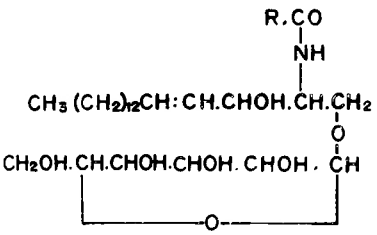


Figure 7.



Cerebrosides

Figure 8.

DISCUSSION

As mentioned previously, cerebrosides (Figure 8) are one of the typical myelin lipids, composing lipoprotein membrane structure of myelin[63)64)] together with other lipids and proteins as was descrbed by FINEAN[21)22)23)24)25)26)27)28)] and others[32)63)64)74)88)93)] through their electron microscopic, biochemical and X-ray difraction studies. Further, considering the observations as presented by LUSE[63)] and De ROBERTIS[16)] and others[83)], that myelin sheath is constructed by invaginated limiting membrane process of oligodendroglia, metabolism of this tissue should be intimately related to the function of oligodendroglia. There have been presented number of investigations[13)14)35)36)65)87)98)99)] concerning the morphological changes in oligodendroglial cells in the edematous subcortical white matter either by light or electron microscope which demonstrated some regressive changes of these cells especially in the late stage of pathological conditions. For this reason some changes had been expected in the metabolism of this lipid in the edematous brain. Inspite of this expectation, however, in the present study, no remarkable changes were observed in the amounts of cerebrosides in the edematous brain, both in the subcortical white matter and brain-stem. On the contrary, in WALLERIAN degeneration, as was reported by JOHNSON[48)49)] and others[66)67)], cerebrosides in sciatic nerve myelin sheath had decreased, but it is a late phenomenon and may not be seen in the acute stage. SEITELBERGER[91)92)] reported, in the demyelinating disease of the cerebrum, cerebrosides still remained[17)] in the perivascular granular cells in the form of granular lipids even in the last stage of the myelin sheath destruction. From these reasons, we may conclude that cerebrosides are inert kinds of myelin lipid and less changeable at least in the acute stage of the brain damage.

Here, there is some discrepancy about the cerebrosides metabolism in the grey matter. As is mentioned before, cerebrosides are lipids of myelin nature and not found in the cell component. Major portion of cerebrosides in the grey matter of the normal brain determined here should be therefore considered to be of myelin nature, which is inevitably mingled into this grossly separated cerebral composition. Increment of cerebrosides in the grey matter of the edematous brain will be discussed later with relation to gangliosides.

Gangliosides (Figure 9)[6)55)57)] are essentially the lipids of nerve cells in the brain[54)56)62)]. Numerous informations could be obtained concerning the importance of this lipid in the cerebral activity[70)71)72)73)101)]. MCILWAIN[70)71)72)73)] had reported the necessity of this lipid or its relating compounds to retain or restore the electrical reactivity of the nerve cell in vitro. WOLFE[106)] disclosed its specific localization in the microsomal fraction[50)84)] particularly in the dendrite and he discussed on the similar localization of synaptic vesicles in subcellular fractionation as investigated by WHITTAKER[34)104)] and others.[15)79)81)82)89)]

From the results of this investigation, presenting decrement of the amounts of gangliosides parallel to the cerebral deterioration indicated by E. E. G. and clinical symptoms, we may consider that 1) gangliosides in some respects concern with the cerebral activity

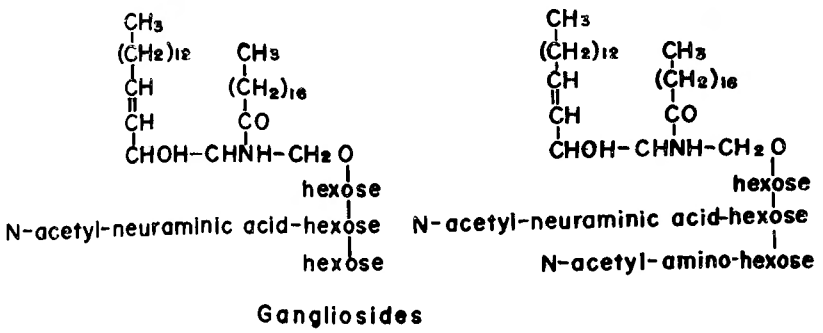


Figure 9.

in vivo, and 2) it is possible that the decrement of this substance will be responsible in part for the deterioration of cerebral activity which was often observed in the case of cerebral edema. As we reported elswhere[2], activation of phospholipids turn-over by acetyl choline, of which mechanism is proposed to concern with the integrity of microsomal structure in the nerve cells as designated by HOKIN[38)39)40)41)], was greatly depressed in the anoxic brain. Considering the local distribution of gangliosides in microsome of the nerve cell[106)] and their probable contribution to construct its membranous structure combined with proteins, interaction of gangliosides with phospholipids turn-over which was suspected to couple with the active transport of sodium ions through the cell membrane[38)39)40)41)], is suggestive and this attracts profound interesting.

It is known that gangliosides show relatively swift metabolic turn-over in the nerve tissue [60)] and are proposed to be catabolized by the *gangliosidase system*[59)], of which enzymatic activities are activated by sequelae products of the degenerated nerve cell elements[52)102)] as demonstrated by KOREY. Increase of cerebrosides in the grey matter of the edematous brain may be well explained in this connection that increased cerebrosides may be the possible denatured products of the gangliosides cleavage through this catabolic enzymatic system.

On the other hand here still remains another interpretation for this problem. It is not unlikely that the nucleoproteins released from the nucleus of the nerve cell during the course of cerebral edema due to the rupture of nuclear membrane have combined with gangliosides to form the compounds not extractable with chloroform-methanol mixtures. It is now known that gangliosides have strong affinity to basic proteins such as histone and protamine[70)71)96)105)] and to make stable complexes which are insoluble to chloroform-methanol mixtures[3)71)73)105)106)]. Migration of nucleoproteins through the nuclear membrane had also been reported in the regressive changes of ganglion cells following the nerve section [21)]. In this connection, however, it is difficult to explain increase of cerebrosides in the grey matter of the edematous brain monistically.

Synthetic pathway of cerebrosides in the brain had been well investigated by BURTON et al[10)] as is outlined in Figure 10.

UDP-galactose + N-acyl sphingosine $\rightarrow$ Cerebrosides

Figure 10. Synthetic pathway of cerebrosides. (Burton, R. M., Sodd, M. A. and Brady, R. O., J. Biol. Chem., 233, 1053 (1958).)

On the other hand, no reports on the biosynthesis of gangliosides have yet been published. However, we may speculate on the synthetic pathway with two routes which are most probable at this time, as were designated by BURTON (Figure 11)[11] In these pathways UDP-glucose will serve as the glucose donor to form gangliosides. UDP-glucose in the brain will be synthesized through the pathays shown in Figure 12, in which UTP is required to form UDP-glucose from glucose-1-phosphate[51)68)77)].

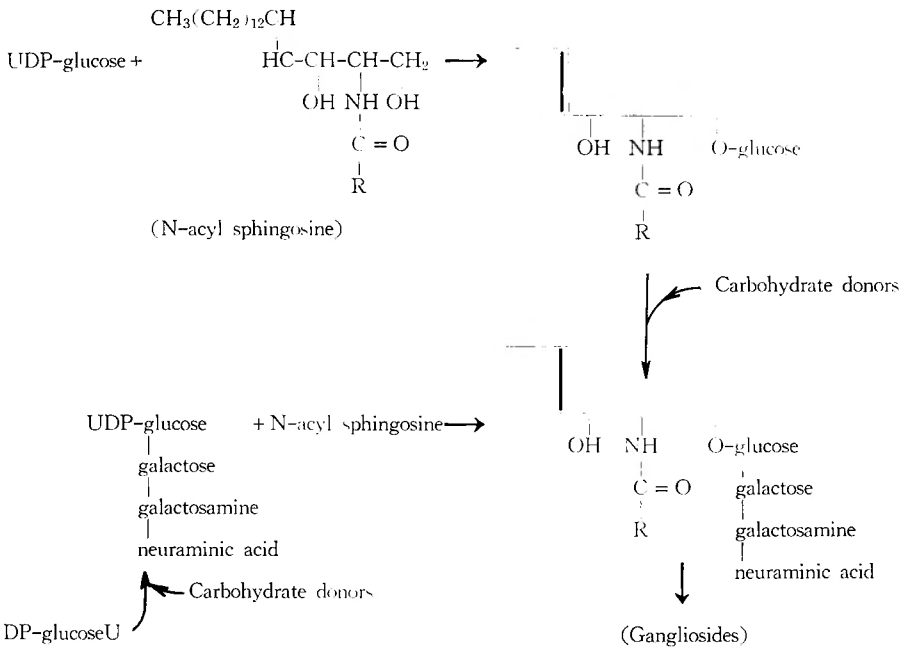


Figure 11. Possible synthetic pathways of gangliosides. (Burton, R. M., in Brady, R. O. and Tower, D. B. (Eds.), The Neurochemistry of Nucleotides and Amino Acids. p. 51 (1959).)

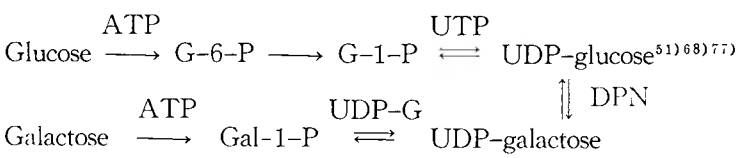


Figure 12.

UTP will be synthesized as follows.

$$UMP + ATP \rightleftharpoons UDP + ADP^{94)}$$

$$UDP + ATP \rightleftharpoons UTP + ADP^{4)5)}$$

or

$$UDP + \text{Phosphoenol pyruvate} \xrightleftharpoons{\text{Pyruvate kinase}} UTP + \text{Pyruvate}^{61)76)100)}$$

Existence of an enzyme capable of phosphorylating uridine to form UMP is proposed from GEIGER's perfusion experiment[31], though it is not proved yet in vitro examination.

From these conceptions we applied uridine derivatives, including uridine (Figure 13), UTP and UDP-glucose (Figure 14) for the therapeutic purpose to the animals with cerebral traumatism. Uridine was most effective in restoring cerebral activity, improving E. E. G.

findings, whereas UTP and UDP-glucose were less effective in the dosis of 20 mg/kg body weight contrary to our expectation.

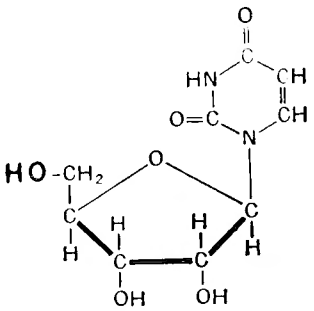
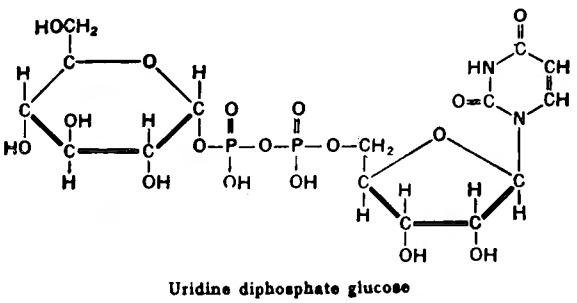


Figure 13. Uridine



Uridine diphosphate glucose

Figure 14.

Matter of penetration of these substances across the Blood Brain Barrier and uptake into the neural cell *in situ* which are relating to the problem of active transport and pinocytosis will take part in this problem. Further investigation is necessary to dissolve the problem.

SUMMARY

1. The concentration of cerebrosides and gangliosides was determined in the grey matter, subcortical white matter and brain-stem of the normal and edematous cat brains at intervals of time 0, 24 and 48 hours after evacuation of epidural balloon content which had been compressing the brain for 24 hours.

2. Wet weight/dry weight retio was determined for grey and subcortical white matter of normal and edematous brains. However, no significant difference in their values between normal and edematous brain was obtained if the free water of the specimen was eliminated by blotting with a bit of filter paper before the measurement.

3. Cerebrosides, one of the typical myelin lipids changed little in the subcortical white matter and brain-stem through the course of 48 hours. However, they slightly increased in the grey matter in the 0 and 24 hours periode specimens.

4. Gangliosides, which virtually exist in the grey matter, particularly in the microsomal fraction which contains cell membranes and dendrites decreased significantly in 0 hour periode specimens and then returned to normal level within 48 hours, as cerebral activity recovered.

5. Uridine derivatives, including uridine, UTP and UDP-glucose which are indispensable for the biosynthesis of gangliosides were adminstered intravenously to the animals with cerebral traumatism for the therapeutic purpose. Among above three, uridine was most effective in restoring the cerebral activity as was evidenced by E. E. G. findings.

6. Results were discussed with reference to the morphological changes of neural cells in the edematous brain, tissue reactivity, *gangliosidase system*, migration of nucleoproteins and also to the problem of penetration of nucleotides through Blood Brain Barrier.

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The gist of the present study has already been reported at the First Asian and Oceanian Congress of Neurology.

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和文抄録

浮腫脳における脂質代謝
実験的脳浮腫における糖脂質代謝

京都大学医学部外科教室第1講座（指導：荒木千里教授）

辻　　宏

1）正常成猫脳及び硬膜外圧迫法により作製した成猫浮腫脳について硬膜外balloonの内容除去後，24時間後及び48時間後の灰白質，皮質下白質及び脳幹部のcerebrosides量及び灰白質中のgangliosides量を測定した．

2）定型的な髄鞘脂質の一種であるcerebrosides量は皮質下白質及び脳幹部では48時間の経過中殆んど変化を示さなかつたが，灰白質では0時間及び24時間標本において軽度の増加が認められた．

3）脳中では主として灰白質，特に神経細胞の細胞膜，dendrites等の属するmicrosomal fractionに含まれるgangliosides量は0時間標本に於いて有意の低下を示したが，脳活性の恢復と共に48時間以内に正常値に復帰する傾向を示した．

4）猫の正常脳及び浮腫脳の灰白質及び皮質下白質のdry weight/wet weight retioを測定したが，測定前，組織中の自由水を濾紙により十分拭去するときは，正常脳と浮腫脳との間に有意の差が認められない事が分かつた．

5）脳浮腫動物(猫)にgangliosidesの生合成に必要なuridine, UTP, UDP-glucose等のuridine誘導物質を治療の目的で投与したが，uridineが脳波所見上から最も有効であつた．

6）以上の結果を脳浮腫におけるneural cellの形態学的変化，組織興奮性の問題，*gangliosidase system*及び核蛋白遊出の問題並びにnucleotidesの脳血液関門通過の問題等に関連して検討論議した．